Appl. No.: 10/620,787

Art Unit: 1648

Reply to Final Office Action of 09/18/2008

Amendment to the Specification:

Please replace paragraphs [0049] and [0051] with the following amended paragraphs:

[0049] Examination of the sequence of L1R-M1R revealed a likely signal sequence at its N-terminus and hydropathy analysis suggested the existence of a membrane spanning domain beginning around position 187. Taking advantage of its natural signal sequence this protein was chosen as the N-terminal component of the polyprotein, thus insuring transport into the lumen of the endoplasmic reticulum and thereby exposing the polyprotein to glycosylating enzymes. Glycosylation can be important to native antigenicity of membrane proteins. This can also promote secretion into the culture medium, simplifying purification. Because neutralizing antibodies are expected to be directed against the extraviral segment of the protein, and to avoid the protein becoming anchored in the membrane, the amino acids after position 186 were not included in the construct. PCR was carried out using vaccinia strain WR as template. The 5' primer added an[[d]] AfIII restriction site and the 3' primer changed the K at position [[176]]177 to the R found in the other sequences (see FIGS. 3 and 9) and added part of a GGGGSSGG spacer-linker sequence following position 186, thereby incorporating a BamHI site near the 3' end of the amplicon. This product was then cloned into the plasmid expression vector PCDNA3.1©(+) (Invitrogen Corporation, Carlsbad, Calif.) between its AfIII and BamH1 sites. This plasmid was prepared by standard means and digested with AfIII and EcoR1 in anticipation of the three-fragment ligation described below.

[0051] A33R-A36R is a type II membrane protein, that is, the N-terminus is cytoplasmic and the C-terminus is external. A hydrophobic sequence that can function as a membrane anchor end at position 57. Amino acids 57 through the C-terminus were included in the construct. In order to incorporate all the variola-specific residues a series of PCR reactions was carried out using a total of 6 oligonucleotide primers. Initially the needed segment of vaccinia was amplified. The 5' most primer completed the

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GGGSSGG spacer-linker sequence that incorporated a BspE1 site at the 3' end of the A33R-A36R construct. Further rounds of amplification were carried out to generate the internal substitutions. In a final round of amplification the 3' most primer added a third GGGSSGG spacer-linker sequence, a 10 histidine tag, and an EcoR1 site. Following digestion with BspE1 and EcoR1 this fragment was combined with the two above in a three-fragment ligation generating an expression vector with a reading frame encoding M1R[[1-86]].186, a GGGGSSGG spacer-linker, A30L, another spacer-linker, A36R57-184, a third spacer-linker, and a histidine tag, under the control of a CMV promoter (see FIGS. 5 and 9). The resultant polyprotein was named [[LMI]LAA (SEQ ID NO:34).